PATENT COOPERATION TREATY

PCT

REC'D 1 9 AUG 2005

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

	Applicant's or agent's file reference PAM-015-PCT			FOR FURTHER	ACTION	See Form PCT/IPEA/416			
International application No. PCT/EP2004/003668				International filing dat 06.04.2004	e (day/month/year)	Priority date (day/month/year) 10.04.2003			
Inte B0	International Patent Classification (IPC) or national classification and IPC B01J19/00, G01N33/551, G01N33/543								
,	Applicant PAMGENE B.V.								
1.	This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.								
2.	This REPORT consists of a total of 6 sheets, including this cover sheet.								
3.	This report is also accompanied by ANNEXES, comprising:								
						ts, as follows:			
		 sent to the applicant and to the International Bureau) a total of 3 sheets, as follows: sheets of the description, claims and/or drawings which have been amended and are the basis of and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 6 Administrative Instructions). 							
		~~,	its which supersede and the disclosure in Diemental Box.	e earlier sheets, but v n the international ap	vhich this Authority con plication as filed, as inc	siders contain an amendment that goes dicated in item 4 of Box No. I and the			
	b. 🗆				indicate type and numb computer readable forr 02 of the Administrative	per of electronic carrier(s)) , containing a nonly, as indicated in the Supplemental anstructions).			
	TI :			<u> </u>					
4.	inis re	port conta	uns indications rela	ting to the following i	tems:				
	⊠ Вох		Basis of the opinion	on					
	□ Вох	No. II	Priority	•					
		No. III	Non-establishmer	nt of opinion with rega	ard to novelty, inventive	step and industrial applicability			
	Вох		Lack of unity of in	vention		, and approaching			
	⊠ Box		applicability, citati	ons and explanations	 with regard to novelty supporting such state 	y, inventive step or industrial ment			
		No. VI	Certain document						
		No. VII		the international app					
☐ Box No. VIII Certain observations on the international application									
Date of submission of the demand					Date of completion of th	ls report			
17.01.2005					17.08.2005				
Name and mailing address of the international preliminary examining authority:					Authorized Officer	Pate.			
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016					Veefkind, V	Supplied Fallows - Figure 19 Annual Parlaments - Figure 19 Annual			
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No. PCT/EP2004/003668

	Box No. I Basis of the repo	ort			
1.	. With regard to the language, siled, unless otherwise indicate	this report is based on the international application in the language in which it wa			
	which is the language of a international search (u	anslations from the original language into the following language, a translation furnished for the purposes of: Inder Rules 12.3 and 23.1(b)) Inational application (under Rule 12.4) Inder examination (under Rules 55.2 and/or 55.3)			
2.	With regard to the elements* of the international application, this report is based on <i>(replacement sheets whic have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):</i>				
	Description, Pages				
•	1-26	as originally filed			
	Claims, Numbers				
	1-17	received on 21.01.2005 with letter of 22.12.2004			
	☐ a sequence listing and/or a	any related table(s) - see Supplemental Box Relating to Sequence Listing			
3.	The amendments have resulted in the cancellation of: ☐ the description, pages ☐ the claims, Nos. ☐ the drawings, sheets/figs ☐ the sequence listing (specify): ☐ any table(s) related to sequence listing (specify):				
4.	☐ This report has been established not been made, since they Supplemental Box (Rule 70.2(c)☐ the description, pages☐ the claims, Nos.☐ the drawings, sheets/fig☐ the sequence listing (sp.☐ any table(s) related to se	s pecify):			
	* If item 4 applies, s	ome or all of these sheets may be marked "superseded "			

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

Yes: Claims

1-17

No: Claims

Inventive step (IS)

Yes: Claims

No: Claims 1-17

Industrial applicability (IA)

Yes: Claims

1-17

No: Claims

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reference is made to the following documents:

D1: WO 02/26376 A (SURMODICS INC) 4 April 2002 (2002-04-04)

D2: WO 99/02266 A (AKZO NOBEL NV ;DAMME HENDRIK SIBOLT VAN (NL); KREUWEL HERMANUS JOH) 21 January 1999 (1999-01-21)

1. The document D1 is regarded as being the closest prior art to the subject-matter of claim 1, and (see passages cited in the Search Report) discloses functionalized substrates, such as functionalized ceramic, functionalized silica or functionalize glass, in which functionalization means addition of organic modification to an inorganic surface, by known methods, to provide bonds with which the photoreactive groups can react (page 9, lines 8-14).

Under the heading "Photoreactive Groups on the Substrate Surface" (bridging pages 10 and 11) an example of this functionalization is given in the form of poly-l-lysine on glass, which is "generally bound to the surface via Silyl-OH groups" (which are the same silyl-OH groups normally found on silica). Then nucleic acid sequences can be printed on then, after which the surface is illuminated (i.e., subjected to electromagnetic irradiation) to cross-link the nucleic acids to the polymer (page 11, lines 9-21). It is used for performing probe-based assays.

On page 9, lines 19-25, the provision of three-dimensional surfaces using a support that is <u>permeable</u> (i.e., implicitly being porous and having through-going channels) to allow nucleic acids to migrate into the pores is described as giving a higher density of nucleic acids.

The subject-matter of the independent claims differs from this disclosure in that the channels are "oriented".

Therefore, these claims are novel over D1 (Article 33(1) and (2) PCT).

- 2.1 The problem to be solved <u>cannot</u> be regarded to be overcoming "the difficulties of polymer coating of inert porous metal oxide substrates" for two reasons:
- a) the qualification "inert" does not appear in the claims. In addition many, if not all, oxides, such as alumina or silica, normally possess many OH groups on the surface, which

are reactive (often acidic, so that interaction with a basic compound would be easy) and can therefore hardly be considered as "inert". It is also mentioned in D1 that these OH-groups are responsible for the fixation of the poly-I-lysine.

- b) the only relevant technical feature in the claims relating to the actual coating of the polymer is "bringing said substrate into contact with a solution comprising said polymer". Apart from being known from D1, this would not appear to overcome much difficulty but rather be a quite obvious step.
- 2.2 The <u>objective</u> problem to be solved must find its origin in the difference with D1. This difference lies in the channels being "oriented".

No surprising effects, resulting from this difference, could readily be identified in the application.

The objective problem to be solved should, thus, be considered as merely providing an alternative permeable metal oxide substrate, suitable for being coated by a polymer for immobilizing biomolecules.

D2 (see passages cited in the Search Report) describes metal oxide substrates with porous, through-going channels for immobilizing biomolecules (particularly electrochemically etched metal sheets are mentioned and more particularly aluminum oxide membranes). It describes a range of advantages associated with these substrates. There is no indication whatsoever in D2 that these substrates would be unsuitable for coating with a polymer.

When wishing to provide an alternative permeable metal oxide substrate, suitable for use in assays and kits, the skilled person would have encountered D2 and, considering the many advantages associated with it, <u>would</u> also have considered it as very suitable alternative substrate for those mentioned in D1.

- 2.3 Thus, none of the independent claims gives rise to an inventive step, contrary to the requirements of Article 33(3) PCT.
- 2.4 None of the dependent claims appears to be able to give rise to an inventive step

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because their features are either already disclosed in D1 or D2, or would (in the absence of specific advantages) be known to be common replacements the skilled person would choose from as a matter of course when wishing to provide an alternative.

Re Item VIII

The independent claims are not in the two-part form in accordance with Rule 6.3(b) PCT, which in the present case would be appropriate, with those features known in combination from the prior art (document D1) being placed in the preamble (Rule 6.3(b)(I) PCT) and with the remaining features being included in the characterising part (Rule 6.3(b)(ii) PCT).

Claims (Retyped)

- 1. A method for providing biomolecules on a porous metal oxide substrate having oriented through-going channels comprising the steps of:
 - a) coating said porous metal oxide substrate with a polymer by bringing said substrate into contact with a solution comprising said polymer such that the polymer in said solution is able to form a coating on a surface of said substrate,
 - b) deposing said biomolecules onto the porous metal oxide substrate obtained in step a) by bringing said biomolecules into contact with said substrate, and
 - c) immobilizing said biomolecules onto the porous metal oxide substrate obtained in step a) by covalently binding said biomolecules to said substrate by means of electromagnetic irradiation.
- 2. A method according to claim 1, wherein said polymer is substantially adsorptively bound on the porous metal oxide substrate.
- 3. A method according to claim 1 or 2, wherein said polymer comprises multiple amide functional groups and/or multiple cationic functional groups.
- 4. A method according to any of claims 1 to 3, wherein said polymer is selected from the group comprising poly-aspartate, poly-glutamate, poly-cysteine, poly-serine, poly-methionine, poly-arginine, poly-histidine, poly-tryptophane, poly-alanine, poly-lysine, poly-leucine, poly-isoleucine, poly-tyrosine, poly-valine, poly-glycine, poly-proline, poly-phenylalanine, poly-threonine, polymers of other natural and non-natural amino acids and derivatives and mixtures thereof.
- 5. A method according to claim 4 wherein said polymer is poly-L-lysine.
- 6. A method according to any of claims 1 to 5, wherein said metal oxide substrate is an aluminium oxide substrate.

- 15. A kit according to claim 14, wherein the label is capable of inducing a colour reaction and/or capable of bio-, chemi- or photoluminescence.
- 16. Method for performing probe-based assays, comprising the steps of:

contacting a sample comprising an analyte to a porous metal oxide substrate having oriented through-going channels and having biomolecules immobilised thereon according to any of Claims 10 to 12;

incubating said sample with said porous metal oxide substrate under conditions suitable for allowing binding of said analyte in said sample to said biomolecules immobilised on porous metal oxide said substrate; and

detecting the binding of said analyte in said sample to said biomolecule immobilised on said substrate.

17. Use of a metal oxide substrate according to any of Claims 10 to 12 for performing probe-based assays.

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- 7. A method according to any of claims 1 to 6, wherein the biomolecules are immobilized on the substrate in spots, thereby forming an array of spots.
- 8. A method according to any of claims 1 to 7, wherein said biomolecules comprise the same or different biomolecules.
- 9. A method according to any of claims 1 to 8 wherein said biomolecules are selected from the group comprising oligonucleotides, polynucleotides, ribonucleotides, proteins, antibodies, antigens, peptides, oligo or poly saccharides, receptors, haptens, ligands, antibodies, antigens, peptides, oligo or poly saccharides, receptors, haptens and ligands, drugs, toxins and liposomes.
- 10. A porous metal oxide substrate having oriented through-going channels obtainable according to the method of any of claims 1 to 9, having a surface that is coated with a polymer, said porous metal oxide substrate having biomolecules immobilised thereon, wherein said biomolecules are immobilised on said substrate by covalent binding by means of electromagnetic irradiation.
- 11. A porous metal oxide substrate according to claim 10, wherein said porous metal oxide substrate is a porous aluminium oxide substrate.
- 12. A porous metal oxide substrate according to claim 10 or 11, wherein said porous metal oxide substrate has a surface that is coated with a polypeptide, and preferably with poly-L-lysine.
- 13. A kit or parts of a kit comprising a porous metal oxide substrate according to any of claims 10 to 12, further comprising a detection means for determining whether binding has occurred between biomolecules and an analyte.
- 14. A kit according to claim 13, wherein the detection means is a substance capable of binding to the analyte and being provided with a label.